



[Home](#) | [This Week's Contents](#) | [ACS Job Bank](#) | [Search C&EN Online](#)

Table of Contents

[ACS Job Bank](#)

[News of the Week](#)

[Cover Story](#)

[Editor's Page](#)

[Business](#)

[Government & Policy](#)

[Science/Technology](#)

[Education](#)

[ACS News](#)

[Calendars](#)

[Books](#)

[Software/Online Briefs](#)

[ACS Comments](#)

[Career & Employment](#)

[Special Reports](#)

[Letters](#)

[Newscripts](#)

[ACS Elections](#)

[Nanotechnology](#)

[What's That Stuff?](#)

[Hot Articles](#)

[Safety Letters](#)

[Chemyclopedia](#)

[Back Issues](#)

[How to Subscribe](#)

[Subscription Changes](#)

[Electronic Reader Service](#)

[About C&EN](#)

[E-mail webmaster](#)

SCIENCE/TECHNOLOGY

November 13, 2000

Volume 78, Number 46

CENEAR 78 46 pp.29-32

ISSN 0009-2347

[\[Previous Story\]](#) [\[Next Story\]](#)

LIGNIN AND LIGNAN BIOSYNTHESIS

*Discovery of proteins that guide phenolic radical couplings
debunks decades-old notions in this field*

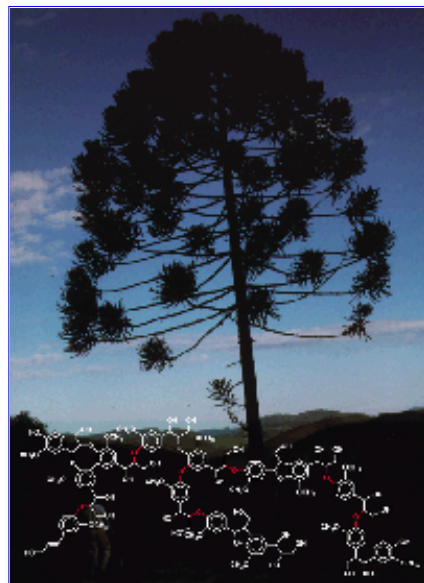
A. Maureen Rouhi
C&EN Washington

The winds of change are blowing in the field of lignin and lignan research.

Lignins are the phenolic polymeric stuff in the woody cell walls of plants. They originate from a metabolic pathway that exists only in land plants. As plants evolved into tall upright forms hundreds of millions of years ago, this pathway evolved to produce lignins that reinforce the plant's cell walls to provide the means to conduct water from the roots to the leaves.

Closely related to lignins are the lignans. These come in various sizes but typically are dimeric. They are found in seed coats, stems, leaves, flowers, and various other plant parts. They generally play a defensive role in the plant.

Lignins are important in the wood industry because they impart a number of desirable qualities to wood, such as strength. But in the paper industry, they are a nuisance, having to be removed from wood fibers for most pulp and paper applications. On the other hand, many lignans are beneficial.



Lignins and lignans are assembled from the same simple phenolic precursors. Understanding how they are formed could have applications in the paper industry, and in the animal feed industry, because low lignin content enhances feed digestibility. The nutraceutical and pharmaceutical industries could exploit the biosynthetic pathways to maximize yields of lignans used to treat or protect against human disease.

Since the 1950s, the prevailing notion of how plants combine lignin and lignan precursors--(*E*)-coniferyl alcohol, (*E*)-*p*-coumaryl alcohol, and (*E*)-sinapyl alcohol--to produce



Related Story

[WHAT'S THAT
STUFF? PAPER](#)

[C&EN, August
14, 2000]

Related People

[Norman G. Lewis](#)

[Simo Sarkanen](#)

[E-mail this article to a
friend](#)

[Print this article](#)

[E-mail the editor](#)

**Lignins enable trees to stand tall.
(Linkages shown in red
predominate in natural lignins.)
[Photo by Laurence B. Davin]**

dimers, oligomers, and polymers was that all it takes is oxidizing capacity to produce radicals from phenolic precursors. At least for the lignins, these radicals then would undergo

random coupling. No other enzymes or proteins were thought to be required.

That notion is now being swept away by findings from the lab of [Norman G. Lewis](#), a professor of plant biochemistry at Washington State University, Pullman, who has been studying lignin and lignan biosynthesis for the past 15 years. At the 22nd International Union of Pure & Applied Chemistry International Symposium on the Chemistry of Natural Products, held in September in Brazil, Lewis described the paradigm shift in this field brought about by the discovery of so-called guiding (dirigent) proteins.

"This random coupling notion didn't make any biochemical sense," Lewis said. Specificity rules in nature. One would expect that the same specificity applies to lignins, nature's second most abundant polymer. Yet five or six different enzymes--all oxidases with broad specificities--have been thought to be involved in lignin formation just because they can oxidize the precursors.

Furthermore, oxidizing capacity alone could not explain the optically active lignans found in nature and the marked regiospecificity observed in lignin formation. With an oxidant such as laccase in vitro, any of the monomers gives a whole range of racemic coupling products, because the radical formed generates many potential coupling sites. "In plants, there is clearly some control in the specificity of this coupling," Lewis said, in the formation of both lignans and lignins.



David (left) and Lewis investigate lignin and lignan formation. [Photo by Maureen Rouhi]

That such control exists was established by the isolation of a protein with no oxidative capacity but with sites that bind either the monomers or the monomer radicals in specific orientations that lead to selective coupling [*Science*, **275**, 362 (1997)]. "We call it a dirigent protein, from the Latin *dirigere*, which means to guide or align," Lewis said of the protein that he discovered three years ago with senior investigator Laurence B.

Davin and others at Washington State and with [Simo Sarkanen](#), a professor of wood and paper science at the University of Minnesota, St. Paul.

The protein was first isolated from *Forsythia intermedia*, a plant that both produces and further metabolizes enantiomerically pure (+)-pinoresinol, a dimeric lignan formed from (*E*)-coniferyl alcohol. An in vitro system containing the protein, an oxidant, and (*E*)-coniferyl alcohol monomers produces (+)-pinoresinol in almost 100% optical purity. A system with only an oxidant and the monomer yields racemic mixtures of random-coupling products. It is still not clear how the protein binds and orients the substrates. "But regardless of the mechanism," Lewis said, "the process is about lining up molecules and enabling only certain types of coupling. That's why we call the protein dirigent."

Additionally, this dirigent protein has a binding site that recognizes only the substrate derived from (*E*)-coniferyl alcohol and not those derived from the two other, structurally related precursors. "That discovery was very exciting for us," Lewis said. It meant that specific binding proteins indeed exist that dictate the outcome of these couplings.

Lewis suspected that other dirigent proteins must exist that engender other distinct coupling modes, such as the coupling that produces only (-)-pinoresinol in flaxseed. Sure enough, he, Davin, and others have found dirigent proteins, as well as homologs, in all major land plant groups that they have examined to date [*Plant Physiol.*, **123**, 453 (2000)].

Furthermore, the Lewis group has cloned the genes encoding the dirigent proteins in *F. intermedia*. Surprisingly, the dirigent proteins have no sequence homology with any other protein of known function [*Chem. Biol.*, **6**, 143 (1999)]. The proteins are new, and they again demonstrate, Lewis said, how gene sequencing doesn't always help identify new biochemical functions. "There wasn't anything in the proteins that would have told you what they do. The guiding function is not something that could have been predicted a priori."

So far, no comparable proteins have been found outside of land plants. This apparent exclusivity to land plants, if confirmed, could have evolutionary implications.



[Photo by Laurence B. Davin

Courtesy of G.H.N. Towers and R.A. Norton

Photo by Laurence B. Davin]

The discovery of dirigent proteins explains the specificity of monomer coupling in lignan biosynthesis. Lewis, Davin, and Sarkanen next proposed that the monomer-specific binding sites in these proteins must be exploited in some way to account for the specificity that is also observed in lignification.

Prior to lignification, layers of cellulosic, hemicellulosic, and proteinaceous components are deposited around the plasma membrane of the cell, defining the boundaries of the wall that will be lignified. The lignin precursors make their way out of the cell through this matrix to precise lignin initiation sites at the farthest end of the wall. Polymerization then occurs at these initiation sites and extends back to the plasma membrane. When lignin biosynthesis is complete, the cell dies.

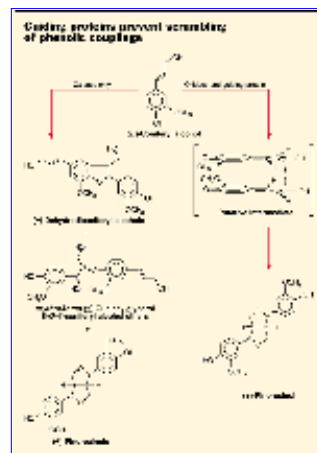
"Nature has developed a remarkable feat of directing lignin monomers to the farthest part of the wall, and that's where macromolecular assembly begins," Lewis said. If oxidation and lignification occurred as soon as the monomers crossed the plasma membrane, a barrier would form. This barrier would restrict or stop the flow of monomers or even engulf the oxidative enzyme. Lignification would stop.

Another of nature's tricks, Lewis pointed out, is that different monomers go to different parts of the wall, and what goes where varies with cell type. "There's no demonstrated scientific evidence establishing how the monomers differentially make their way across the wall and then polymerize back," he said.

Yet another puzzle is that, in nature, a specific linkage predominates that is different from the dominant type found in synthetic lignins. That is, a regiospecific preference exists naturally. "What we like to think is that, if lignans form under careful control, then lignification involves a comparable process," Lewis said. "Discussions with Sarkanen led to the recognition that proteins with arrays of monomer-specific binding sites can provide that control."

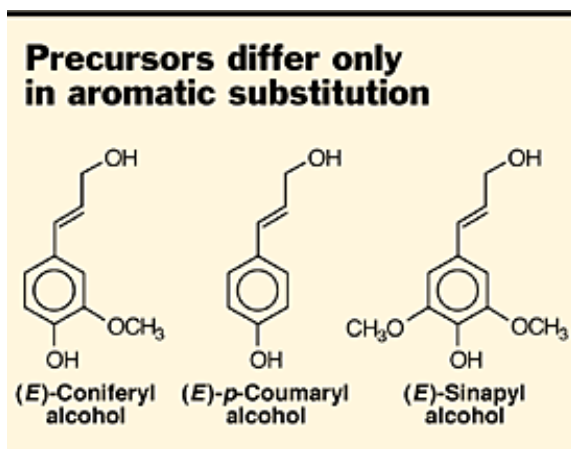
The finding that dirigent proteins are associated with lignifying cells, among others, has prompted Lewis, Davin, and Sarkanen to propose the following

hypothesis: Lignin formation is controlled by proteins containing an array of dirigent (monomer-specific binding) sites similar to those that individually control lignan biosynthesis, but which are located near the lignin initiation sites of specific cell types. The proteins dictate the monomers that will be incorporated and the type of linkage that will be formed through the specificity and orientation of their binding sites. They assemble a progenitor macromolecule from the monomers through a series of oxidations and couplings. Once the progenitor is formed, it is replicated by a direct template mechanism.



Sarkanen, Lewis said, has demonstrated template polymerization in vitro.

Lewis also pointed out that lignin precursors are polymerized directly into lignins during lignification. No buildup of intermediates occurs. This fact was not recognized earlier, he said, because oligomeric lignans were thought to be low-molecular-weight lignins. It is clear now that lignins and lignans are products of distinct metabolic pathways.



And if the lignin precursors were to be replaced with some other phenolic monomers, the substitutes would be unable to undergo template polymerization to form lignins. For example, transgenic plants with abnormal monomer composition cannot make lignins properly, Lewis noted. Instead, they form substances that do not replicate the structural

properties of natural lignins. These findings provide "excellent evidence in accord with our model for arrays of dirigent sites and template polymerization," Lewis said.

"That's where we are now," Lewis concluded. The questions that couldn't be answered before--how the specificities are occurring--must be tied up with dirigent proteins and arrays of dirigent sites. The Lewis group hopes to identify the nature of the binding sites and eventually discover the mechanisms involved in lignin and lignan biosynthesis. ◀

[\[Previous Story\]](#) [\[Next Story\]](#)

[Top](#)

[Home](#) | [Table of Contents](#) | [News of the Week](#) | [Cover Story](#)
[Business](#) | [Government & Policy](#) | [Science/Technology](#)

Chemical & Engineering News

Copyright © 2000 American Chemical Society - All Right Reserved
1155 16th Street NW • Washington DC 20036 • (202) 872-4600 • (800) 227-5558

